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THE DETECTION OF HYPOCHLORITES AND CHLORAMINS IN MILK AND CREAM.

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The use of hypochlorites and chloramins in the treatment of dairy utensils has led to much speculation as to whether or not attempts might be made to add these preparations to milk. This speculation is due to the fact that there have been no methods for detecting small amounts of chlorin in milk. The term "chlorin," as used in this paper, refers to the available chlorin derived from hypochlorites and chloramins.

OUTLINE OF EXPERIMENTAL WORK.

Very small quantities of chlorin in water solution can easily be detected, either by means of the iodid-starch or the *o*-toluidin test. However, when applied to milk containing small amounts of chlorin, these tests are of no value, because the chlorin has combined with the protein of the milk and is not liberated readily at room temperature by the addition of acids.

It seemed desirable, therefore, to devise some methods for the demonstration of small amounts of hypochlorites or chloramins in milk and cream. The question first arose, how firmly is the chlorin united with the protein? If loosely combined, there was no reason why it should not be liberated under proper conditions.

On experiment it was found that the chlorin could be liberated; and if liberated in the presence of potassium iodid, the chlorin would displace the iodin of the iodid, which would then combine with the protein, giving rise to a yellow-colored compound, the shade of the color depending on the amount of iodin liberated. If, however, only small amounts of iodin were liberated, then the colored protein compound would be masked by the large excess of unaltered protein. The small amount of colored protein could then be made to react with starch, producing a blue color which could be more readily discerned, thus making the test more delicate.

In order to determine in what dilution the hypochlorites and chloramins can be detected in milk and cream a series of solutions, each containing 100 cubic centimeters of milk or cream plus definite amounts of hypochlorite or chloramin solution, were prepared, so that the final results were solutions containing chlorin from 1:1,000 to 1:100,000 parts of milk or cream. The flasks containing these solutions were placed in the ice box for 24 hours and then examined for chlorin. After standing at room temperature for several hours, the flasks were returned to the ice box, and again examined after 48 hours. A flask of plain milk was added as a control.

PREPARATION OF REAGENTS.

(*a*) *Potassium iodid solution.*—Dissolve 7 grams of potassium iodid in 100 cubic centimeters of distilled water. Prepare fresh.

(*b*) *Hydrochloric acid.*—To 100 cubic centimeters of concentrated hydrochloric acid add 200 cubic centimeters of distilled water.

(*c*) *Starch solution.*—Boil 1 gram of starch in 100 cubic centimeters of distilled water. Cool before using.

THE TEST.

(1) To 5 cubic centimeters of milk or cream in a medium-sized test tube add 1.5 cubic centimeters of the iodid solution, mix thoroughly by shaking, and observe the color of the milk.

(2) If unaltered, add 4 cubic centimeters of hydrochloric acid, mix thoroughly by means of a glass rod flattened at one end, and note the color of the curd.

(3) Next place the tubes in a large water bath, previously heated to 85° C., and allow to remain for 10 minutes. During this interval the curd will have risen to the surface.

(4) The tubes are then cooled rapidly by placing in cold water, and when cold are examined for any color change that may have taken place, either in the curd or in the liquid below.

(5) Finally, add 0.5 to 1 cubic centimeter of starch solution to the liquid below the curd and note the result.

REACTION IN MILK.

Milk containing 1 part of chlorin in 1,000 parts of milk acquires a distinct reddish color, while at 1:2,500 dilution it is still slightly discolored when compared with the control. This holds good for both hypochlorites and chloramins.

In case the milk contains an amount of hypochlorite corresponding approximately to 1 part of chlorin in 5,000 parts of milk, then the milk acquires a pale-yellow color on the addition of the iodid solution, and the color deepens as the amount of chlorin increases, so that at 1:1,000 dilution the milk has a yellowish-brown color. At 1:5,000 dilution the yellow color is not permanent and fades gradually. If kept at room temperature it is necessary to add hydrochloric acid at 1:5,000 dilution in order to bring out the yellow color. With chloramins a yellow color is produced at 1:2,500 dilution. If less chlorin is present than mentioned above, the milk retains its natural color.

In more dilute solutions of hypochlorites and chloramins it is necessary to heat the milk-iodin-hydrochloric-acid mixture in order to bring about the reaction. The cooling is necessary to prevent a too rapid decomposition of the iodid by the excess of hydrochloric acid.

With hypochlorites and chloramins the curd and the solution below acquire a distinct pale-yellow color with 1 part of chlorin in 50,000 parts of milk. The shade of the color varies somewhat and is a little paler with chloramins than with hypochlorites. The liquid below the curd is also colored yellow, the shade corresponding to the depth of color of the curd.

When milk which has stood at room temperature for 24 hours is treated as above, the coloration produced is a little paler than when kept at ice-box temperature.

The curd of the control which has been kept at ice-box temperature shows only a slight yellowish tinge when compared with the curd of a blank which contains no iodid. The liquid below the curd in the control, however, is most distinctive, since it shows no trace of yellow when compared with the blank containing no iodid.

The addition of starch to the liquid below the curd brings out the reaction somewhat more prominently by changing the pale-yellow color to a light-reddish purple, the coloration passing through red purple into a deep-blue purple as the amount of the chlorin present increases. The control is also slightly discolored by the addition of starch and may acquire a light-straw color, but without any trace of a red-purple tint.

In milk which has been kept at room temperature for a long time, the control may have a stronger straw color, but without any trace of purple. On this account it is best to judge the presence of small amounts of hypochlorites and chloramins by the color of both the curd and the liquid below.

TABLE 1.—*The hypochlorite test in tabular form.*[1]

Test.	Parts of available chlorin with different color reactions.					
	1 : 1,000	1 : 2,500	1 : 5,000	1 : 10,000	1 : 25,000	1 : 50,000
Take 5 c.c. milk........	Reddish color.	Slightly discolored.	....	....	....	....
Add 1.5 c.c. iodid solution. Milk appears.	Yellowish brown.	Deep yellow.	Pale yellow, fades.	....	....	....
Add 4 c.c. HCl. Curd appears.	Yellowish brown.	Deep yellow.	Light yellow	....	....	....
After heating at 85° C. and cooling, the curd and liquid below appear.	Yellowish brown.	Deep yellow.	Yellow......	Yellow...	Pale yellow.	Yellowish.
Add 0.5 c.c. starch solution. Liquid below curd appears.	Blue purple.	Blue purple.	Blue purple.	Dark-red purple.	Red purple.	Pale-red purple.

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[1] The test for chloramins is approximately the same.

REACTION IN PASTEURIZED MILK.

If hypochlorite solution is added to milk in different proportions and the milk is then pasteurized at 145° F. for 30 minutes, the results obtained by the above test after the milk has been kept in the ice box for 24 hours are approximately the same as with raw milk.

REACTION IN CREAM.

Hypochlorites or chloramins added to 20 per cent cream in different proportions give results approximately the same as with milk.

With 1 part of chlorin in 2,500 parts of cream, the cream turns yellow on adding the iodid solution. At 1:5,000 dilution and above, no change is apparent after adding hydrochloric acid to the cream-iodid mixture, and it is necessary to heat the cream in order to bring about the reaction. At 1:25,000 dilution both the curd and the liquid below are a pale yellow, while at 1:50,000 dilution the curd is a little more yellow than the control and the liquid below is the same as in the control. After adding starch to the liquid below the curd, it acquires a pale-red purple color with hypochlorites at 1:50,000 dilution, while with chloramins it remains the same as the control at this dilution. At 1:25,000 dilution the reaction is distinct with chloramins.

After heating the cream-iodid-hydrochloric-acid mixture, the presence of small amounts of chlorin must be judged by the color of the liquid below the curd, because the curd, owing to the presence of the fat globules, has a pale-yellowish color. The yellow color of the curd becomes more marked as the amount of chlorin present increases, while the liquid below the curd has the same color as in milk.

The above test applies equally well after the milk or cream has been kept in the ice box for 48 hours.

Before applying the above test for the detection of chlorin in milk or cream, it is advisable to prepare a series of flasks containing hypochlorite solution in different proportions in order to become acquainted with the coloration produced.

The test becomes more delicate by using 2 cubic centimeters of the iodid solution, but has the disadvantage that the controls of certain old milks give a slight reaction on the addition of starch.

SUMMARY.

1. A test for the detection of hypochlorites and chloramins in milk and cream by means of potassium iodid and hydrochloric acid has been described.
2. One part of chlorin in 50,000 parts of milk or cream can be detected by this method.
3. Milk kept in the ice box for 24 and 48 hours still gives the reaction.
4. Milk kept at room temperature for 24 hours gives the reaction, but a little less marked than if kept cold.
5. Milk pasteurized, after the addition of hypochlorites, at 145° F. for 30 minutes reacts the same as raw milk.
6. Twenty per cent cream gives the reaction in approximately the same degree as raw milk.
7. It is best to judge the presence of added hypochlorites or chloramins by the color of both the curd and the liquid below the curd.

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